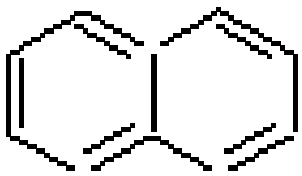
American Chemistry Council Naphthalene Panel
American Chemistry Council Hydrocarbon Solvents Panel
American Coke and Coal Chemicals Institute
American Petroleum Institute Regulatory Analysis and Scientific Affairs
Utility Solid Waste Activities Group
Western States Petroleum Association



***ad hoc* Naphthalene Coalition**

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Office of Environmental Health Hazard Assessment
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Oakland, CA 94612

Re: Comments Opposing OEHHA's Proposed Unit Risk Factor for Naphthalene

Dear Dr. Salmon:

This letter responds to the solicitation of public comments by the Office of Environmental Health Hazard Assessment (OEHHA) on the *Proposal for the Adoption of a Unit Risk Factor for Naphthalene* dated January 23, 2004 (the Proposal). These comments are submitted on behalf of the American Chemistry Council (ACC) Naphthalene Panel, the ACC Hydrocarbon Solvents Panel, the American Coke and Coal Chemicals Institute (ACCCI), the American Petroleum Institute (API) Toxicology Task Force, the Utility Solid Waste Activities Group (USWAG) and the Western States Petroleum Association (WSPA) (collectively the *ad hoc* "Naphthalene Coalition" or "Coalition").[1]

Members of the Naphthalene Coalition are national and regional industry associations that represent a broad spectrum of the regulated community in California and around the country. Coalition members together represent more than 500 chemical and petroleum manufacturers, distributors, users, and electric power generation and natural gas utilities whose daily operations involve the safe handling of naphthalene. Accordingly, our member companies share a common commitment to ensure that principles of sound science and consistency shape federal and state guidelines and standards involving naphthalene.

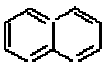
It is from this perspective that the Naphthalene Coalition calls into question OEHHA's proposed unit risk factor for naphthalene. First, the Coalition questions the prudence of OEHHA's decision to move forward with the Proposal when the United States Environmental Protection Agency (USEPA) is poised to release for external peer review its draft federal assessment of naphthalene cancer endpoints, including reference doses (RfDs) and reference concentrations (RfCs), for the Integrated Risk Information System (IRIS). According to USEPA, the announcement of the naphthalene peer review panel is expected by April; the peer review of the IRIS assessment is expected to be completed shortly thereafter, but no later than June 2004. Given the near completion of the USEPA's work on naphthalene risk factors, it

[1] *See* Attachment A, Descriptions of Members of the Naphthalene Coalition.

makes no sense for OEHHA to move forward without the benefit of EPA's work. Moreover, OEHHA's decision to act before the completion of USEPA's IRIS assessment is contrary to the State of California's long-standing policy of coordinating with USEPA on the development of risk assessments, including a memorandum of understanding between OEHHA and USEPA on the coordinated development of risk assessments on molecules of shared concern. *See* discussion in section 1 below. In short, OEHHA's failure to consider USEPA's naphthalene review ignores an important national peer-reviewed analysis of the current science on naphthalene. It is also contrary to state policy and increases the likelihood that OEHHA's unit risk factor will be inconsistent with the federal risk factors and thereby unnecessarily increasing the burden and cost of regulatory compliance in California.

OEHHA's decision to move forward with the Proposal ahead of USEPA's pending review is particularly troubling given significant shortcomings in the evidence and flaws in OEHHA's analysis underlying the proposed unit risk factor. These flaws, discussed in detail in sections 2 through 7 below, include the following:

- *inappropriate use of a linear model for deriving the unit risk factor when the weight of scientific evidence supports the conclusion that naphthalene is not genotoxic.* The Proposal assumes low dose linearity in estimating the unit *risk factor* for naphthalene. However, this assumption is contrary to the weight of the scientific evidence, including a large number of genotoxicity and metabolism studies, which find that naphthalene is *not* genotoxic, either directly or through metabolites. The evidence does not support linearity in the low dose range and, therefore, the use of a linear model for deriving a unit *risk factor* for naphthalene is not appropriate;
- *use of a model that is inconsistent with naphthalene's likely mode of action.* The Proposal fails to recognize the many studies that suggest a cytotoxic mode of action for naphthalene. In light of these studies, OEHHA needs to consider alternative models that are consistent with the more likely mode of action of naphthalene;
- *combined use of unrelated tumor types to calculate the unit risk factor.* The unit *risk factor* calculations in the Proposal are predicated on the improper combination of incidence rates for two unrelated tumors: Nasal Respiratory Epithelial Adenomas (NREA) and Nasal Olfactory Epithelial Neuroblastoma (NOEN). This is not an appropriate method to conduct a quantitative potency calculation as a basis for calculating a unit *risk factor*;
- *calculation of a unit risk factor without appropriately including all relevant data on rats from the NTP studies.* The improper exclusion of data on rats from NTP studies distorts the statistical analysis and extrapolation that is the basis for the proposed unit *risk factor*;
- *use of a model that is not a statistically significantly fitting model (i.e., the quantal linear model) to calculate unit risk, while other more appropriate models are available (e.g., a quantal-quadratic model); and*
- *failure to recognize that the linear multistage model does not adequately fit the data.*



Accordingly, the Naphthalene Coalition respectfully urges OEHHA to withdraw the Proposal and reevaluate it after the results of USEPA's IRIS assessment of naphthalene (including external-peer review comments) can be integrated into the OEHHA review. When the peer reviewed IRIS assessment of naphthalene can be incorporated into OEHHA's review, the Coalition requests that OEHHA reevaluate the Proposal in light of the IRIS assessment and the Coalition's comments in sections 2 through 7 below prior to seeking review by the Scientific Review Panel (SRP).

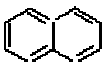
If OEHHA chooses to forward the Proposal to the SRP without waiting for the release of USEPA's peer reviewed work, then the Coalition requests that OEHHA apprise the SRP of the status of USEPA's peer review so that the SRP can consider the pendency of EPA's work in making a final recommendation on the Proposal. Also, if OEHHA chooses to move forward with the Proposal regardless of the scientific flaws discussed in sections 2 and 3 below, then the Coalition requests that the unit risk factor be corrected to reflect the calculation errors identified in sections 4 through 7.

1. OEHHA SHOULD CONSIDER USEPA'S UPCOMING PEER-REVIEWED ASSESSMENT OF NAPHTHALENE.

USEPA's IRIS program is in the final stages of a detailed assessment of cancer risk factors associated with naphthalene. According to USEPA, peer review of the assessment is expected to be completed by June this year. Further, the pesticidal uses of naphthalene are currently being evaluated, and subsequent risk assessments are being prepared as part of the USEPA reregistration program. Based on these extensive assessments – which span years of study evaluation and review - and the technical information presented below, OEHHA should not proceed with a unilateral effort to develop a unit risk factor for naphthalene, but instead, should collaborate with USEPA's IRIS evaluation, and work towards a harmonized assessment of naphthalene.

Such coordination is required under the 1996 Memorandum of Understanding (MOU) between California's Environmental Protection Agency (Cal/EPA) and the USEPA entitled *Memorandum of Understanding Between California EPA's Office of Environmental Health Hazard Assessment and the U.S.EPA's National Center for Environmental Assessment.* The MOU is intended to foster harmonization of the State and federal risk assessment programs, to reduce the potential for conflicting approaches and methods, to exchange work products, and to share resources more efficiently. The MOU was entered, in part, due to the parties' joint recognition that diminishing government resources necessitated better and more extensive coordination among federal and state agencies engaged in risk analyses. Given the State of California's fiscal crisis and the diminishing resources available to the USEPA, the utility of the MOU finds no better example than in its application here, which would compel OEHHA to suspend work on naphthalene until the IRIS assessment for naphthalene is completed.

The MOU is only one example of a long history of coordination between the USEPA and the State of California. For example, OEHHA's 1997 policy entitled *Improving the Scientific Basis of Risk Assessment Through Harmonization* confirms the importance the State of California places on coordinating with the USEPA and the need to conserve scarce resources.



See http://www.oehha.org/risk/raac/harmadv.html. The policy states that harmonization of State and Federal risk assessment activities should be viewed as a two-way exchange of scientific analysis, methods, and approaches. The policy specifically notes that:

> it has become increasingly apparent that different organizations using divergent risk assessment methodologies for the same chemical or comparable situations creates a difficult situation for risk managers, policy makers and stakeholders alike.

OEHHA states in the policy that harmonization should be viewed as "[m]aking the most effective use of our limited resources by information sharing."

As a further example, the USEPA's Office of Pesticide Programs and California's Department of Pesticide Regulation have a long history of joint review and coordination on pesticide registrations. This collaboration has proved to save time and resources, and has minimized risks of developing inconsistent risk results or duplicative risk assessment procedures that challenge risk assessors and regulators alike.

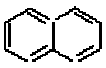
Harmonization of risk assessment work was one important theme of the Risk Assessment Advisory Committee (RAAC), an external advisory Committee that reviewed the risk assessment practices of Cal/EPA.[2] RAAC made the following recommendation to the Cal/EPA in its final report:

> Cal/EPA should endeavor to develop future risk assessments in concert with US EPA, especially for high volume and/or high risk compounds. Before Cal/EPA conducts an independent risk assessment for a substance, it should first review any existing US EPA risk assessment.

Naphthalene clearly falls within the description of a "high volume" compound. On March 3, 2004 the Coalition asked USEPA if the Agency could estimate when the IRIS peer review panel on naphthalene would convene. USEPA responded that the external peer review would likely convene in the next few months. Given the imminent timing of USEPA's IRIS peer review of naphthalene, which is expected to include a unit risk factor for naphthalene, OEHHA should coordinate with USEPA to develop its unit risk factor in concert with USEPA.

In light of the State of California's policy of coordinating with USEPA on the development of risk assessments, the imminent release of the IRIS peer review assessment on naphthalene, and OEHHA's MOU with USEPA on coordinating the development of risk assessments on molecules of shared interest, OEHHA should suspend work on naphthalene and resume it only after the results of the IRIS assessment of naphthalene can be integrated into the OEHHA review. All of the foregoing policies and practices, and the need for sensible regulation and fiscal prudence, require no less.

[2] The Risk Assessment Advisory Committee, comprised of 34 nationally known scientists with expertise in the discipline of risk assessment, was a response to Senate Bill 1082 (Calderon), which called for the Director of Cal/EPA's Office of Health Hazard Assessment to appoint a panel of distinguished experts to review Cal/EPA's risk assessment procedures.



2. OEHHA's USE OF LOW DOSE LINEARITY TO DETERMINE A HUMAN UNIT RISK FACTOR IGNORES THE CURRENT WEIGHT OF EVIDENCE ON NAPHTHALENE.

The Proposal uses the default assumption of low dose linearity because OEHHA believes the weight of the evidence "favors the interpretation" that the carcinogenicity of naphthalene is due to a reactive metabolic intermediate, which causes direct damage to DNA. The Proposal states:

> Genetic toxicology results for naphthalene are mixed: *Salmonella* reverse mutation assays were generally negative, but some test results with eukaryotic systems *in vivo* or *in vitro* were positive (National Toxicology Program (NTP), 2000). However, it was considered on balance that the weight of evidence, including metabolism to 1-naphthol via an epoxide intermediate (NTP, 1992, citing Bock *et al.*, 1976 and others; NTP, 2000), and the reactivity of naphthoquinones to cellular components (Zheng *et al.*, 1997) favors the interpretation that the mechanism of naphthalene carcinogenicity likely involves a reactive metabolic intermediate which causes direct damage to DNA. A low dose linearity assumption is therefore appropriate when extrapolating from the point of departure to obtain an estimate of the cancer risk at low doses.[3]

This analysis simply ignores the considerable weight of evidence on naphthalene from other studies. The evidence as a whole does not support an assumption of low dose linearity. Both the genotoxicity and the metabolism of naphthalene have been extensively evaluated, as summarized below. The Naphthalene Coalition believes the weight of the scientific evidence favors the interpretation that the tumorigenic effects of naphthalene do not involve genotoxicity or direct damage to DNA. Therefore, the use of a low dose linear model for deriving a human risk factor for naphthalene is inappropriate.

a. Naphthalene and its metabolites should not be considered in overly broad comparisons of genotoxicity and carcinogenicity associated with Polynuclear Aromatic Hydrocarbons (PAHs)

The draft naphthalene health effects summary document states (page 2, paragraph 2) that, "if information about the carcinogenicity of naphthalene had been available at the time, the carcinogenicity of naphthalene would have been evaluated in conjunction with benzo(a)pyrene and other carcinogenic PAHs."

While some chemists would agree that naphthalene can be technically classified as a PAH for purposes of definitional nomenclature, the importance of PAHs as a group is associated with their biological activity. Biologically active PAHs share a common mechanism for genotoxicity and carcinogenicity based on their structure, which allows for metabolic conversion via the cytochrome P450 enzyme, CYP1A1, to an active dihydrodiol-epoxide.

Although planar fused ring compounds (PAHs) vary considerably in their biological activity, genotoxic PAHs are indirect-acting or promutagens, such that genotoxicity is only

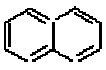
[3] Proposal at page 14.

expressed following metabolic conversion of the PAH to an active species. The mechanism by which PAHs are thought to induce tumor formation is via interaction with genetic material within target cells, either frank mutagenicity or interference with normal genetic biology as a result of PAH-adduct formation with nuclear material. Accordingly, it is generally observed that the genotoxic potency of PAHs closely parallels the carcinogenic potency. However, this relationship is based on experience with PAHs having greater than two fused rings and information suggests that the active structure of some PAHs is a reactive arene oxide, in older literature termed the bay region diol-epoxide. A bay region diol-epoxide is formed in a PAH when *three rings* are fused in a way to create a pocket, the "bay". Bay region diol epoxides are formed enzymatically in humans by CYP1A1. The ability and ease of a PAH to form a bay region diol epoxide can be calculated. This has led to a great deal of work in structure-activity-relationship (SAR) assessment of the potential for carcinogenicity of PAH compounds - but only PAH compounds with three or more fused rings.

In addition to the recognition of the importance of the bay region to genotoxicity and carcinogenicity of PAHs, it has been observed that the addition of a substituent group, almost always a methyl group, in or opposite to the bay region containing the epoxide impacts on PAH biologic activity. There are numerous examples of the alkylation of the PAH (with a methyl group) both enhancing and eliminating PAH tumorigenicity and mutagenicity (Saas, 1996; Slaga, 1979, Thakker, 1979).

Naphthalene is both biologically and structurally distinct from the biologically active genotoxic and carcinogenic PAHs. Naphthalene does not have greater than two fused rings and, as discussed below, the weight of evidence supports the conclusion that naphthalene is not mutagenic. Unlike the PAHs, the metabolism of naphthalene is under the control of the CYP2F enzyme family, not the CYP1A family, and does not lead to the formation of a dihydrodiolepoxide but instead form naphthalene-1,2-oxide. Further, the large and long-standing body of information relating to carcinogenic characteristics of PAHs, whether it be induction or suppression of genotoxic/ carcinogenic activity, has not been associated with naphthalene.

To date, a unified SAR theory does not exist to account for the observations of PAH carcinogenicity, particularly for PAHs that are substituted beyond the methyl state (nitro-aromatics and branched chain alkylated PAHs, for instance). Various illuminating bodies of work have evaluated the carcinogenic effect of methyl-, ethyl-, and propyl-substitutions on fused-ring PAHs such as chrysene. Methylation has been shown to transform inactive PAHs to active and to de-active carcinogenic PAHs. For example, methylchrysene is a more potent lung carcinogen than chrysene, but ethyl- and propyl-chrysene are less potent. Similarly, bay region methylation of dimethylbenzanthracene, a potent mutagen and carcinogen, completely blocks mutagenic and carcinogenic activity. However, none of these observations characteristic of PAH carcinogenicity have been found applicable to "PAHs" with less than three fused rings. In fact, no approach to PAH carcinogenic SAR, whether involving electron cloud density theories or methods of analysis involving statistics and artificial intelligence, includes naphthalene in the paradigm.



b. The weight of evidence strongly supports the conclusion that naphthalene is not genotoxic

The results of the genotoxicity studies of naphthalene are primarily negative, and reviews by others of these data support this interpretation. Recently, the genotoxicity of naphthalene encompassing both the published literature and several unpublished studies was reviewed (Schreiner, 2003). The review contained results from 16 bacterial assays, 9 cytogenetic assays (7 *in vitro*, 2 *in vivo*) and 13 other assays, including 6 cell transformation assays, 3 unscheduled DNA synthesis assays, 2 alkaline elution assays, one *Drosophila* assay, and a human cell gene mutation assay. Tables summarizing the data presented in Schreiner (2003) are included here as Attachment B. Naphthalene did not induce positive responses in any of the 30 *in vitro* assays conducted with nonmammalian and mammalian cells and gave negative results in all 4 assays in which animals were directly dosed and evaluated. Positive responses were only seen in 4 *in vitro* assays: the NTP chromosomal aberration assay in CHO cells, an *in vitro* micronucleus assay in a human lymphoblastoid cell line, a mouse embryo chromosome assay and the *Drosophilia* assay. It should be noted that two of the negative *in vivo* assays were micronucleus assays.

The genotoxicity of naphthalene has also been considered by at least four national and international agencies over the last few years.

1. The authors of the NTP study report (NTP, 2000 at page 20) stated:

   > There is little evidence for mutagenic potential of naphthalene in the most widely used genotoxicity assays.

2. USEPA's Toxicological Review of Naphthalene (USEPA, 1998 at page 24), developed in support of its IRIS database, concluded:

   > The available data suggest that the genotoxic potential of naphthalene and/or its metabolites is weak, at best

3. The monograph documenting the review of naphthalene by the International Agency for Research on Cancer (IARC, 2002 at page 418) states:

   > There is little evidence for induction of gene mutations by naphthalene. In contrast, positive results were obtained in assays for micronucleus formation, chromosomal aberrations, and chromosomal recombinations *in vitro*, which are consistent with a clastogenic potential.

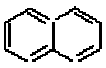
4. In the European Union (EU) Risk Assessment Report for Naphthalene (EU, 2003 at page 155) document, it was concluded:

   > Overall, the balance of evidence indicates that naphthalene is not genotoxic.

Collectively, the weight of evidence strongly favors the interpretation that naphthalene is not genotoxic.

c. Studies indicate that naphthalene metabolites are not genotoxic

The metabolism of naphthalene has been extensively investigated and reported in the literature. It has been demonstrated that naphthalene metabolites are responsible for the cytoxicity noted in the respiratory tract in mice and rats (Buckpitt *et al.*, 1992; Buckpitt *et al.*, 1995; Plopper *et al.*, 1992). The primary step in the metabolism of naphthalene in mammalian

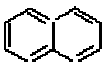


species is oxidation, catalyzed by cytochrome P450 oxygenases (CYP2F family) to naphthalene-1-2-epoxide; both the 1R2S and 1S2R enantiomers may be formed. The epoxide has a very short half-life of 3.6 minutes (Buonarati *et al.*, 1989) and may spontaneously rearrange to form naphthols (primarily 1-naphthol) and eventually form naphthalene diols and naphthoquinones. The epoxide can also be enzymatically conjugated with glutathione by glutathione S-transferases to form a variety of glutathione conjugates that are excreted in the urine as n-acetylcysteine. Naphthalene-1,2-epoxide can also be enzymatically hydrated by epoxide hydrolase to form naphthalene-1,2-dihydrodiol, which can be conjugated with sulfate and glucuronic acid, or converted to naphthalene-1,2-dihydrodiol by catechol reductase, forming naphthoquinone via oxidation (USEPA, 1998). Further hydroxylation of naphthols, catalyzed by microsomal O2/NADPH2-dependent monooxygenases, results in naphthalenediols which, via enzymatic and autocatalytic oxidation, form 1,2- and 1,4-naphthaquinones.

The genotoxicity studies of naphthalene also evaluated the metabolites of naphthalene; the results do not support the conclusion that the metabolic products of naphthalene are mutagenic. Naphthalene metabolism and the potential for metabolites to induce genotoxicity were considered in many of the genotoxicity evaluations included in the Schreiner (2003) review. The majority of the *in vitro* genetic toxicology tests included a "metabolic activation" component. Many compounds are not mutagenic or carcinogenic but can be converted to mutagens (promutagens) or carcinogens (procarcinogens) by mammalian metabolism (Casarett and Doull, 1995). Unlike *in vivo* assays, the short term *in vitro* assays require exogenous metabolic activation to detect promutagens. The most common means to provide metabolic activation is the addition of a postmitochondrial supernatant from a rat liver homogenate (S9 mixture). The results of the *in vitro* genotoxicity assays with metabolic activation were generally negative. Further, rat hepatocytes, which are metabolically active, were evaluated in two *in vitro* unscheduled DNA synthesis assays and an alkaline elution assay. Naphthalene was not mutagenic in these assays. *In vivo* assays, which permit the metabolism of naphthalene, were also negative. The weight of evidence from these studies supports a conclusion that naphthalene metabolites produced *in situ* do not result in a mutagenic response. This conclusion is supported by mutagenicity studies conducted with naphthalene metabolites *per se*.

In several studies, the naphthalene metabolites, 1-naphthol and 2-naphthol, were not mutagenic in *S. typhimurium* with or without metabolic activation (Florin *et al.*, 1980; McCann *et al.*, 1975, Narbonne *et al.*, 1987). Further, naphthoquinone was not mutagenic in several strains of *S. typhimurium* with or without metabolic activation (Sakai *et al.*, 1985). Flowers-Geary *et al.* (1994) reported that naphthalene-1,2-dione was mutagenic in strains of *S. typhimurium* without metabolic activation. The naphthalene metabolite 1-naphthol failed to produce positive results in several other genotoxicity assays, including tests for sex-linked recessive lethal mutations in *D. melanogaster* (Gocke *et al.*, 1981), mutations in mouse L5178Y cells (Amacher and Turner, 1982), unscheduled DNA synthesis in cultured rat hepatocytes (Probst and Hill, 1980) and induction of micronuclei in bone marrow cells in mice (Gocke, 1981) and rats (Hossack and Richardson, 1977) after *in vivo* exposures.

In consideration of the above, the assumption that naphthalene is genotoxic is contrary to the weight of evidence and at variance with conclusions reached by federal and international government agencies that have recently reviewed naphthalene. The underlying basis for



assuming low dose linearity to determine the human unit risk factor is seriously flawed and should be reconsidered. The weight of the scientific evidence does not favor the interpretation that the mechanism of naphthalene carcinogenicity likely involves a reactive metabolic intermediate which causes direct damage to DNA. Therefore, it is not appropriate to assume low dose linearity in estimating a unit risk factor for naphthalene. The Naphthalene Coalition thus urges OEHHA to withdraw the Proposal and consider alternative models that are consistent with the most likely mode of action of naphthalene, as discussed in the next section.

3. USE OF A GENOTOXIC RATHER THAN A CYTOXIC MODEL TO EVALUATE MODE-OF-TUMORIGENIC-ACTION FOR NAPHTHALENE IS INCONSISTENT WITH THE FINDINGS OF KEY MECHANISTIC STUDIES.

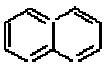
Mode of action for induction of tumors is a key element in contemporaneous carcinogen risk assessment (USEPA, 2003). Mechanistic studies have been conducted in experimental animals and tissues to determine the mode of action for naphthalene toxicity and possible carcinogenicity (Buckpitt *et al.*, 1992; Buckpitt *et al.*, 1995; Plopper *et al*., 1992). Overall, these studies suggest a cytotoxic mode of action for naphthalene.

In the NTP mouse study (NTP, 1992), exposure-related increases in the incidences of chronic inflammation, metaplasia of the olfactory epithelium and hyperplasia of the respiratory epithelium were the predominant nonneoplastic changes observed. Neoplastic findings were limited to an increased incidence of alveolar/bronchiolar adenomas in female mice exposed at the highest concentration (30 ppm) only.

In rats, non-neoplastic lesions were seen in the nasal tissues of both males and females (NTP, 2000). The findings consisted of hyperplasia, atrophy, chronic inflammation and hyaline degeneration of the olfactory epithelium and hyperplasia, squamous metaplasia, hyaline degeneration and goblet cell hyperplasia of the respiratory epithelium. Neoplastic findings were limited to these tissues. Exposure-related increases in respiratory epithelial adenomas and in olfactory epithelial neuroblastomas were seen in both sexes of rats.

a. Site and species differences in naphthalene toxicity correlate with higher rates of metabolism in mouse lung and rat nasal tissue

The NTP studies and the published literature support the conclusion that varying high-dose exposures to naphthalene cause cellular injury and increased cell replication rates in the ciliated and the Clara cells of the bronchiolar epithelium in mice and in the nasal epithelium of rats and mice. Intraperitoneal administration of naphthalene produces injury (swelling, vacuolization, exfoliation and necrosis of the tracheobronchial epitheal Clara cells of mice but not rats (Plopper *et al.*, 1992). In this same study, naphthalene was also cytotoxic to the olfactory epithelium of both rats and mice; however, the effects in mice occurred at much higher doses than rats, which suggests increased sensitivity of the nasal tissues in rats. These site and species differences in toxicity correlate well with higher rates of metabolism by mouse lung tissue and rat nasal tissue. Investigation of metabolism by lung or liver microsomes demonstrated that metabolism of naphthalene to a covalently bound protein product and to 1-naphthol and naphthalene-1,2-dihydrodiol was 10-fold greater in mouse tissue than in rat tissue.



The ratio of 1-naphthol to 1,2-dihydrodiol in mouse lung was 17-fold higher than in mouse liver (Buckpitt *et al.*, 1984; Tingle *et al.*, 1993). Buckpitt *et al.* (1992) characterized the stereochemistry of naphthalene epoxidation in preparations of nasal mucosa, lung and liver of mouse, rat, hamster and monkey. The highest metabolic rates were observed in mouse lung and liver microsomal incubation mixtures: rat, hamster and monkey lung preparations metabolized naphthalene at 12%, 37% and 1%, respectively, of the rate in mouse lung. Murine microsomal fractions were characterized by an excessive, stereospecific formation of the 1R,2S-naphthalene epoxide from naphthalene with 1R,2S:1S,2R ratios of 10:1 to 30:1 in incubations with lung microsomes and 1:1 to 5:1 in liver microsomes depending on the initial naphthalene concentration. In lung microsomal preparations from rat, hamster and monkey, enzymes yielded 1R,2S:1S,2R ratios of 0.48, 0.61 and 0.12, respectively. Subsequent investigation of the role of cytochrome P-450 (CYP) monooxygenases in the mouse lung demonstrated that CYP 2F2 catalyzes the stereospecificity of naphthalene metabolism to 1R,2S-oxide in nonciliated cells at all airway levels and is a critical determinant of species-specific and region-specific cytotoxicity of naphthalene in mice (Buckpitt *et al.*,1995; Shulz *et al.*, 1999). Since mice are prone to developing alveolar/bronchial adenomas, continuous damage to Clara cells by chronic naphthalene exposure and subsequently high levels of 1R,2S-epoxide could stimulate increased expression of these tumors.

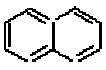
In the rat, an obligate nose breather, the olfactory epithelium contains the greatest amounts of CYP protein of all tissues studied in the rat (Baldwin *et al.*, 2004). As noted for the mouse lung, cytotoxicity is most prevalent in tissues with the highest capacity for metabolizing naphthalene. Thus, the high levels of CYP protein in the olfactory epithelium of the rat may explain the sensitivity of this tissue to the cytotoxicity from naphthalene exposure. It is postulated that the significant cell damage in the rat nasal epithelium is followed by cell proliferation and repair, which frequently includes mutational events secondary to the induced toxicity and unrelated to direct genotoxicity. Based on the metabolic differences in this tissue, the tumors are considered species specific as no nasal tumors were noted in exposed mice.

b. Primates have low pulmonary metabolic capacity for naphthalene

The observations may also have relevance to man. *In vitro* metabolism studies of naphthalene using pulmonary tissue fractions from humans and nonhuman primates show that the metabolic capacity is 1 to 2 orders of magnitude lower than that in rodents. Further, recent research shows that the nasal epithelium of nonhuman primates contains levels of CYP2F, the primary microsomal enzyme involved in naphthalene metabolism in the rat nasal epithelium, that are roughly *10- and 20- fold less* than the corresponding tissues in rats and mice, respectively.

Collectively, these results strongly demonstrate that tumors induced by naphthalene are related to the metabolic capacity of the specific tissue and that ultimate induction of cytotoxicity in that tissue potentially leads to a tumorigenic event. Current research suggests that the metabolic capacity of the lung and nasal tissues of humans and nonhuman primates are an order of magnitude lower than the rodent species and, therefore, these tissues may not be susceptible to the effects of naphthalene.

In light of the relevant metabolic differences between rodent species and humans, the Naphthalene Coalition urges OEHHA to withdraw the Proposal and reconsider the mode of turmeric action using alternative models that are consistent with the likely cytotoxic mode of



action of naphthalene. Ideally, this model should account for differences in metabolism and tissue susceptibility between rodents and humans.

4. IT IS NOT APPROPRIATE TO COMBINE UNRELATED TUMOR TYPES TO CALCULATE A UNIT RISK FACTOR.

The Proposal combines Nasal Respiratory Epithelial Adenomas (NREA) and Nasal Olfactory Epithelial Neuroblastoma (NOEN) incidence rates to calculate a unit risk factor for naphthalene. Although NREA and NOEN were considered together in NTP's conclusion of "Clear Evidence for Carcinogenicity," this was based on a weight of evidence approach. It is **not** appropriate for OEHHA to combine NREA and NOEN for the purposes of a quantitative potency calculation as a basis for proposing the naphthalene unit risk factor. The tumor types should not be combined because they are pathologically unrelated.

OEHHA has developed and adopted guidelines for calculating cancer potency factors based on animal data.[4] These guidelines specifically address the issue of combining tumors as follows (OEHHA, 2002 at page 13):

> Where both benign and malignant tumors are induced ***at the same site*** and the malignant tumors are significantly increased, the data on both types of tumors may be combined to form the basis for risk assessment. [emphasis added]

The tumor types combined in the Proposal are not tumors "at the same site." Although NREA and NOEN occur in the same general region of the body, they are histologically distinct, and they are certainly not induced "at the same site." By analogy, liver adenoma and lung carcinoma occur in the same general region of the body (*i.e.*, the abdominal cavity); however, it would not be appropriate to combine these tumors because they do not occur "at the same site."

When combining tumors, it is also important to consider whether benign tumors have the potential to progress to the associated malignancies of the same histologic origin. Benign and malignant tumors are combined only when the benign tumor is expected to progress to the malignant tumor observed at an increased incidence. OEHH (2002 at page 9) has described USEPA's approach to combining benign and malignant tumors as follows:

> US EPA separates tumor incidence data according to organ sites or tumor types. The incidence of benign and malignant tumors is combined whenever scientifically defensible. US EPA considers this incidence combination scientifically defensible ***unless the benign tumors are not considered to have the potential to progress to the associated malignancies of the same histogenic origin***. [emphasis added]

Based on these criteria, it is inappropriate to combine the tumor types in the rat bioassay of naphthalene. There is no reasonable basis for concluding that NREA has the potential to progress to NOEN. The two tumor types are histologically distinct and unrelated; one cannot progress to the other. Therefore, NREA and NOEN should not be combined to calculate a cancer potency factor for naphthalene, as in the Proposal.

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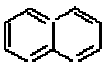
[4] OEHHA. (2002) Air Toxics Hot Spots Program Risk Assessment Guidelines. Part II. Technical Support Document for Describing Available Cancer Potency Factors. December, 2002.



5. THE DATA USED FOR THE CALCULATIONS DID NOT INCLUDE ALL OF THE RATS FROM THE NTP STUDIES AND, THEREFORE, THE SUBSEQUENT CALCULATIONS ARE INCORRECT.

The calculations in the Proposal excluded certain animals within the exposure groups, so subsequent calculations are incorrect.

Table A
Exposure, number of animals on test, and response

Chamber Concentration (ppm)	Average Concentration (mg/m^3)	Animals on Test (NTP,2000)	Animals used in OEHHA Analyses	Animals with Epithelial Adenoma
0	0	49	44	0
10	9.67	49	42	6
30	29.0	48	44	8
60	58.0	48	41	15

The Proposal considered only animals alive after the first occurrence of a tumor (see footnote b in Table 3 of the Proposal). As a result, fewer animals were included in their analyses. This practice is incorrect for at least two reasons:

1. ***Exclusion of animals within the exposure groups from the statistical analyses violates the assumptions of the statistical models.***

   The statistical analyses used in the Proposal assume that exposure groups are statistically independent. If the number of animals in each group is based on the animals alive after the first occurrence of a tumor, then the number of animals in each group is dependent on the common value of the first occurrence of a tumor and therefore the groups are not independent. The assumption of independence for the statistical analyses (LMS and BMD) is fundamental, and if the assumption is not met the resulting calculations are incorrect.

2. ***Exclusion of animals within the exposure groups creates a non-random sampling.***

   If the result of the animal study is to be extrapolated to humans, the sampling scheme has to be similar. A complete random sample of animals is the only reasonable choice because there is not a corresponding selection of humans where you only consider the tumor rate in humans after the appearance of the first tumor.

An often-stated reason for using the reduced animal count is that it accounts for differences in survivorship. In the current analysis the survivorship was not different among the groups (2nd full paragraph, page 8 of the Proposal). Therefore, there is no compelling reason to use the reduced numbers in the analyses. In fact, reducing the sample size for the calculations produces incorrect results.

6. THE BENCHMARK DOSE (BMD) ANALYSIS DID NOT USE THE OPTIMUM MODEL.

There are two key problems with the BMD analysis presented in Table 6 of the Proposal. First, the Naphthalene Coalition assessed the modeling and found that an optimum fitting quantal-linear model could not be determined. This is illustrated in Attachment C, which shows that one of the terms in the model could not be estimated and was replaced by a boundary value. Second, the fitted model was not statistically different from a mean model. In other words, the model is not effective (it yields a p-value of 0.4943). As seen in Attachment D, the model still does not adequately fit the data even if all of the animals in each group are analyzed (p-value of 0.5166).

The third column of Table 6 of the Proposal indicates the epithelial adenomas analysis model fit was adequate. These values describe the differences between observed and predicted values, but not if the model is statistically different from a mean model (a model that predicts only the mean without considering exposure).

The quantal-quadratic model is the best-fit model for the data, as seen in Attachment E ($p<0.016$). In this model all parameters are estimated by the data and the LED_{10} is 25.30 mg/m^3. A similar analysis with the reduced data set has a model fit p value of 0.0156 and a LED_{10} of 23.00 mg/m^3.

Using the optimum LED_{10} value 25.30 mg/m^3, the estimated human unit risk factor would be 0.017 $(mg/m^3)^{-1}$.

In summary, the quantal linear model used in the Proposal is not a statistically significantly fitting model, whereas the quantal-quadratic model is a statistically significantly fitting model and estimates a human unit risk value of 0.017 $(mg/m^3)^{-1}$.

7. THE LINEAR MULTISTAGE (LMS) MODEL DOES NOT ADEQUATELY FIT THE DATA.

The Naphthalene Coalition ran the LMS analysis using MSTAGE with the reduced and full data sets and found that a full model fit was not possible because the LMS model requires that the coefficients be non-negative. This is similar to the problems in the fitting of the quantal-linear model in Table 6 of the Proposal (noted above). The two statistical models (the LMS and quantal-linear) are similar in mathematical form.

To test if the fitting problems were unique to the MSTAGE computer program the data and LMS model were fit using other computer programs such as TOXRISK and an LMS program from Crump Associates. These programs were unable to estimate terms in the LMS model with these data because the programs failed to converge. Thus, these results indicate the LMS model is not appropriate for these data.

Note that the value 0.01919 as the q_{animal} estimate for the epithelial adenomas analysis in Table 5 of the Proposal is incorrect. Based on a reassessment of the reduced data set, the value should be 0.01191 (the upper confidence limit on parameter 1; see Attachment F), which corresponds to a human unit risk factor of 0.018 $(mg/m^3)^{-1}$. If the full data set is used with this

incorrect method the value of q_{animal} should be 0.01015 with a corresponding human unit risk factor of 0.016 $(mg/m^3)^{-1}$.

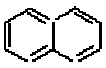
In summary, the LMS model does not adequately fit the data. If the LMS model is applied in spite of this, the resulting human unit risk factor is 0.016 $(mg/m^3)^{-1}$.

CONCLUSIONS

For all the foregoing reasons, the Naphthalene Coalition urges OEHHA to withdraw the Proposal and reevaluate it after the IRIS assessment has been released. At a minimum, the Naphthalene Coalition requests that OEHHA hold the proposal in abeyance and not forward it to the SRP until USEPA's external peer review document on naphthalene is released, which is expected to be in June, a few short months from now. If OEHHA nonetheless proceeds without the external peer review document, the Naphthalene Coalition requests that in forwarding the Proposal to the SRP, OEHHA apprises the SRP of the status of USEPA's peer review so that the SRP can consider the imminent release of this document in making a recommendation on the Proposal.

The Naphthalene Coalition further urges OEHHA to properly factor the weight of evidence as discussed above and to correct calculation errors. If OEHHA chooses to move forward with the Proposal regardless of the scientific flaws discussed in sections 2 and 3 above, then the Naphthalene Coalition requests that the unit risk factor be corrected to reflect the calculation errors identified in sections 4 through 7.

* * * * * * *



The members of *ad hoc* Naphthalene Coalition appreciate the opportunity to provide our views on the proposed unit risk factor for naphthalene. We look forward to an opportunity further to explore the scientific basis of the proposed value. In the meantime, if the Coalition can provide any further assistance or information, please contact Dr. Anne P. LeHuray at (703) 741-5630 or *anne_lehuray@americanchemistry.com*.

Sincerely yours,

American Chemistry Council Hydrocarbon Solvents Panel and the American Chemistry Council Naphthalene Panel
Courtney M. Price, Vice President CHEMSTAR

American Coke and Coal Chemical Institute
David C. Ailor, P.E., Director of Regulatory Affairs

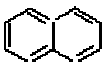
American Petroleum Institute Regulatory Analysis and Scientific Affairs
Howard Feldman, Director of Regulatory Analysis & Scientific Affairs

Utilities Solid Waste Activities Group
Joseph E. Shefchek, Chairman

Western States Petroleum Association
Michael Wang, Manager, South Coast Region, Toxics, Legal, Pipeline and Tax

cc: Dr. Joan E. Denton, Director, OEHHA
Mr. Val F. Siebal, Chief Deputy Director
Dr. George Alexeef, Deputy Director for Scientific Affairs, OEHHA
Dr. Melanie A. Marty, Chief, Air Toxicology and Epidemiology Section, OEHHA

Attachments:
Attachment A: Members of the *ad hoc* Naphthalene Coalition
Attachment B: Summary of Available Genetic Toxicity Studies for Naphthalene
Attachment C: BMD Analysis, Quantal Linear, Reduced Data Set
Attachment D: BMD Analysis, Quantal Linear, Full Data Set
Attachment E: BMD Analysis, Quantal Quadratic, Full Data Set
Attachment F: MSTAGE Output, Full Data Set



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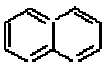

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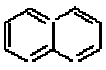

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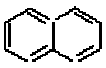


Attachment A
Members
of the
ad hoc Naphthalene Coalition

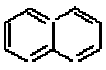
The Hydrocarbon Solvents Panel and the Naphthalene Panel of the American Chemistry Council (ACC) represent the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and security performance through Responsible Care®, common sense advocacy designed to address major public policy issues, and health and environmental research and product testing. **The ACC Hydrocarbon Solvents Panel** represents producers of aliphatic and aromatic hydrocarbon solvents and provides product stewardship for users of hydrocarbon solvents. **The ACC Naphthalene Panel** represents naphthalene producers through advocacy, communication and research focused on naphthalene.

The American Coke and Coal Chemicals Institute (ACCCI) represents independently owned and operated "merchant" companies that produce metallurgical coke (both furnace and foundry coke); integrated steel companies that produce metallurgical coke; producers and processors of chemicals derived from the distillation of coal and coal tar; coke sales agents; and, suppliers to these producers and processors.

The American Petroleum Institute (API) is the primary trade association for the oil and natural gas industry in the United States. Representing one of the most technologically advanced industries in the world, its membership includes more than 400 companies engaged in all aspects of the oil and gas industry, including the exploration, production, refining, transportation and marketing of crude petroleum and petroleum products. API is a major research institute that advances public policy positions based upon scientific, technical and economic research, and it develops standards and quality certification programs used throughout the world. API's public policy positions reflect a commitment to ensure a strong, viable U.S. oil and natural gas industry capable of meeting the energy needs of our nation and providing consumers a reliable source of products in an efficient and environmentally responsible manner.

The Utility Solid Waste Activities Group (USWAG) is an association of the Edison Electric Institute (EEI), the American Public Power Association (APPA), the National Rural Electric Cooperative Association (NRECA), the American Gas Association (AGA), and approximately 80 electric utility operating companies located throughout the country. EEI is the principal national association of investor-owned electric power and light companies. APPA is the national association of publicly-owned electric utilities. NRECA is the national association of rural electric cooperatives. AGA is the national association of natural gas utilities. Together, USWAG members represent more than 85 percent of the total electric generating capacity of the United States and service more than 95 percent of the nation's consumers of electricity and over 93 percent of the nation's consumers of natural gas.

The Western States Petroleum Association (WSPA) is trade association representing nearly 30 companies that explore, develop, refine, market and transport petroleum and petroleum products in the western United States.



Attachment B

Summary
of
Available Genetic Toxicity Studies
for
Naphthalene

Tables 1, 2 and 3 from

Schreiner, C.A. 2003. Genetic Toxicity of Naphthalene: A Review.
J. Toxicol. Env. Health, Part B, 6:161-183

TABLE 1. Naphthalene Genetic Toxicology: Bacterial Systems

Assay Type	Organism	Doses[1]	Results	Reference
Bacterial Mutation	Sal. typhimurium plate incorp. ±rat S9 TA1535, TA1537, TA100, TA98	Naphthalene 100µg/plate	Negative <70 revertants/plate	McCann et al., 1975
		1-naphthol 1000µg/plate	Negative <70 revertants/plate	
	Sal. typhimurium Plate incorp. ±rat and hamster S9 TA1535, TA1537, TA100, TA98	0.3-100µg/plate	Negative toxic at max dose	Mortelmans et al. 1986
	Sal. typhimurium Plate incorp. ±rat and hamster S9 TA1535, TA1537, TA100, TA98	0.3-100µg/plate	Negative toxic at max dose	NTP, 1992
	Sal. typhimurium Plate incorp. ±rat S9 TA1537, TA1538	10-200µg/plate	Negative; toxic above 100µg/plate	Gatehouse, 1980
	Sal. typhimurium Taped plate assays for volatiles ±rat S9 TA100, TA98	10-50µg/plate	Negative	Bos et al, 1988
	Sal. typhimurium. Plate incorp. ±rat S9 TA1535, TA1537, TA100, TA98	0.03-30µmole/plate toxic >3µmole/plate	Negative	Florin et al., 1980
	Sal. typhimurium. ±rat S9 TA1535, TA1537, TA100, TA98	250µg/plate Naphthalene, Naphthoquinone	Negative	Sakai et al, 1995
	Sal. typhimurium. ±rat S9 TA1535, TA1537, TA1538, TA100, TA98	3-300µg/plate	Negative, toxic above 300µg/plate	Godek, 1985 Stankowski, 1987 (details in Table 4)

TABLE 1 (cont)

Assay	*Organism*	*Doses*[1]	*Results*	*Reference*
Bacterial Mutation	Sal. typhimurium TM677 (8-azaguanine resistant) ±rat S9	1-2mM	Negative	Kaden et al., 1979
	Sal. typhimurium TA98, TA 1535 ±rat S9	Naphthalene, 1-naphthol: 5- 1000 µg /plate	Naphthalene and 1-naphthol negative at 1000µg /plate in both strains. Naphthalene weakly positive in TA1535 at 5, 10µg /plate, no dose response	Narbonne et al, 1987
	Sal. typhimurium UTH8414, 8413 TA100, TA98. ±rat S9	100-2000µg/plate	Negative	Conner et al., 1985
SOS Response	Sal typhimurium TA1535/p5K1002 (uMuC-lacZ) ±rat S9	83µg/ml	Negative	Nakamura et al., 1987
	E. coli K12 inductest (λ lysogen GY5027; uvrB$^-$,envA$^-$) quantitative plate test ±rat S9	2000µg/plate	Negative	Mamber et al, 1984
SOS Chromotest	E. coli PQ37 (sfiA::lacZ fusion). ±rat S9 (50%standard mix)	0.156 –10.0µg/assay	Negative	Mersch-Sundermann et al, 1993
E. coli rec assay	WP2/WP100 (uvrA$^-$, recA$^-$) suspension assay ±rat S9	2000µg/ml	Negative	Mamber et al., 1983
E. coli pol assay	WP2/WP67 (uvrA$^-$, polA$^-$) ±rat S9	none given	Negative	Mamber et al., 1983
	WP2/WP3478 (polA$^-$) ±rat S9	none given	Negative	

[1] Doses identifies dose range or highest inactive dose.

TABLE 2. Naphthalene Genetic Toxicology: Cytogenetic Systems

Assay Type	*Test Method*	*Organism*	**Doses[a]**	*Results*	*Reference*
Cytogenetics in vitro	Sister chromatid exchange (SCE) (Litton Bionetics, Inc.)	Chinese hamster ovary cells (CHO) ± rat S9 (Aroclor 1254 induced)	-S9: 9-90µg/ml; 26 hr exposure +S9: 2.7-27µg/ml; 2-hr exposure (2 trials)	Positive (2nd trial only) - S9 at 27-90µg/ml; +S9, at 15, 27µg/ml	NTP, 1992 (results considered negative by UK HSE)
	SCE (Univ. Liverpool, Dept Pharmacol. & Therapeutics)	Human peripheral mononuclear leukocytes (MNL) ± human liver microsomes	100µM (13µg), 2 hr exposure; 72 hr harvest	Negative for SCE, mitotic and proliferative indices ± human microsomes; cytotoxic+ microsomes	Tingle et al., 1993 Wilson et al, 1995
	SCE (Univ. Liverpool, Dept. Pharmacol. & Therapeutics)	Human peripheral mononuclear leucocytes (MNL) ± human liver microsomes	10- 100µM (1.3 –13ug), 2hr exposure; 72 hr harvest naphthalene-1,2-dihydrodiol naphthalene epoxide 1-naphthol 1,2 and 1,4-naphthoquinone	1,2 –dihyrodiol and epoxide negative for SCE and cytotoxicity; 1-naphthol - cytotoxic + microsomes; naphthoquinones- positive for SCE –microsomes and cytotoxic	Wilson et al, 1996
	Micronucleus (MN): CREST assay (Univ. Calif., Riverside, CA	Human B-lymphoblastoid cells MCL-5	Naphthalene 40ug/ml 1,4 naphthoquinone 0.1ug/ml	Positive: chromosome breakage-type MN Positive: chromosome loss-type MN.	Sasaki et al, 1997
	Chromosome aberrations (Litton Bionetics)	Chinese hamster ovary cells (CHO) ± rat S9 (Aroclor 1254 induced)	-S9, 15-75 (8-10hr exposure; 10.1 & 20.5 hr harvest; +S9, 30-67.5µg/ml (2 hr exposure; ~20.5 hr harvest)	Positive +S9 at 30-67.5µg/ml; cell cycle delay	NTP, 1992

TABLE 2 (cont)

Assay Type	*Test Method*	*Organism*	**Doses[a]**	*Results*	*Reference*
	Chromosome aberrations (Texas A&M Univ., Vet Anatomy Dept & TEES Engin Toxicol. Div.)	Preimplantation whole mouse embryos (72 hr post-conception) ± rat S9	0.16mM	Positive; 10 fold inc. –S9; 30 fold inc. +S9, slightly embryotoxic	Gollahon et al, 1990 (abstract only)
Cytogenetics in vivo	Micronucleus assay	ICR-1 Swiss mice, male	50, 250, 500mg/kg single oral gavage	Negative at 24 hr sacrifice	Harper et al, 1984
	Micronucleus assay (Pharmakon Res. Intern'l)	CD-1 mice, male and female	250mg/kg single intraperitoneal	Negative at 30, 48, 72 hr sacrifices, toxic>250mg/kg	Sorg, 1985 (see details in Table 5)

a- Doses identifies dose range, highest soluble dose or highest inactive dose.

TABLE 3. Naphthalene Genetic Toxicology: Other Systems

Assay Type	Organism	Doses[a]	Results	Reference
In vitro cell transformation	High passage Fischer rat embryo cells, F1706P96	0.1, 0.5µg/ml	Negative	Freeman et al., 1973
	Syrian baby hamster kidney cells (BHK-21C13) + rat S9 (Aroclor induced)	0.08-250µg/ml	Negative	Purchase et al., 1978
	Human diploid fibroblasts (WI-38) + rat S9 (Aroclor-induced)	0.08-250µg/ml	Negative	Purchase et al., 1978
	Mouse (BALB/c) whole mammary gland cultures	0.001-1.0µg/gland	Negative cytotoxic above 0.1µg based on gland regression and absence/paucity of alveolar buds	Tonelli et al., 1979
	BALB/c-3T3 mouse cell culture	15-150µg/ml; max. conc. based on 10-20% cell survival	Negative toxic at highest dose	Rundell et al., 1983
In vivo neoplastic transformation	F344 partially hepatectomized rats (sex not specified)	100mg/kg in corn oil, single oral dose	Negative for gamma glutamyl transpeptidase foci	Tsuda et al., 1980
Gene mutation in human cells	Human B-lymphoblastoid cell line MCL-5 (hprt and tk loci)	Naphthalene 40µg/ml 1,4-naphthoquinone 0.1µg/ml	Negative	Sasaki et al, 1997

TABLE 3 (cont)

Assay Type	Organism	Doses[a]	Results	Reference
Unscheduled DNA Synthesis (UDS)	Primary rat hepatocytes in vitro	0.5-1000nM/ml; 1-naphthol 2-naphthol, only	Both negative at 100nM/ml, highest non-toxic dose	Probst et al., 1981
	Primary rat hepatocytes in vitro	0.16-5000µg/ml	Negative Toxic above 16µg/ml	Barfknecht, 1985 (details in Table 6)
	Primary hepatocyte cultures from rats treated in vivo	600, 1000, 1600mg/kg single oral gavage dose	Negative No toxicity	RTC, 1999 (details in Table 7)
Alkaline Elution	Rat hepatocytes in vitro	3mM; 3 hr exposure	Negative for increased incidence of DNA single strand breaks	Sina et al., 1983
	Hepatocytes from treated female Sprague Dawley rats	359mg/kg oral (1/5 LD50) at 21 and 4 hrs prior to sacrifice	Negative for DNA single strand breaks in hepatocytes; dose inhibited liver GSH	Kitchin et al, 1992, 1994
Drosophila melanogaster	Somatic mutation and recombination (SMART assay)	1, 5, 10mM in feed of larva for 48 hrs until pupation	Positive dose dependent loss of heterozygosity of 2 recessive wing genes (mwh, flr)	Delgado-Rodriguez et al., 1995

a- Doses identifies dose range, highest soluble dose or highest inactive dose.

Attachment B
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Attachment C
BMD Analysis, Quantal Linear, Reduced Data Set

```
 ================================================================
        Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16
$
        Input Data File: D:\MYSAS\CALEPA\NAPHTHALENE.(d)
        Gnuplot Plotting File:  D:\MYSAS\CALEPA\NAPHTHALENE.plt
                                          Sat Feb 28 09:32:26 2004
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]


   Dependent variable = COLUMN2
   Independent variable = COLUMN1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0111111
                          Slope =   0.00774744
                          Power =            1   Specified


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Background    -Power
                  have been estimated at a boundary point, or have been
specified by the user,
                  and do not appear in the correlation matrix )

                   Slope

     Slope            1
```

Attachment C (cont)
BMD Analysis, Quantal Linear, Reduced Data Set

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	0.00843096	0.00157481

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

This indicates the model could not be fully fitted properly

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-65.0122			
Fitted model	-66.2104	2.39643	3	0.4943
Reduced model	-77.8454	25.6665	3	<.0001

AIC: 134.421

This indicates model fit is not statistically significant

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	44	0
9.6700	0.0783	3.288	6	42	1.558
29.0000	0.2169	9.544	8	44	-0.5647
58.0000	0.3868	15.857	15	41	-0.2748

Chi-square = 2.82 DF = 3 P-value = 0.4201

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 12.4969

BMDL = 9.3263

Estimated LED_{10}

Attachment D
BMD Analysis, Quantal Linear, Full Data Set

```
 ================================================================
        Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
        Input Data File: D:\MYSAS\CALEPA\NAPHTHALENE.(d)
        Gnuplot Plotting File:  D:\MYSAS\CALEPA\NAPHTHALENE.plt
                                          Sat Feb 28 09:39:36 2004
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]


   Dependent variable = COLUMN2
   Independent variable = COLUMN1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =         0.01
                          Slope =   0.00638318
                          Power =            1   Specified


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Background    -Power
                    have been estimated at a boundary point, or have been
specified by the user,
                    and do not appear in the correlation matrix )

                     Slope

     Slope            1
```

Attachment D (cont)
BMD Analysis, Quantal Linear, Full Data Set

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	0.00718653	0.00134022

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

This indicates the model could not be fully fitted

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-69.6561			
Fitted model	-70.7955	2.27867	3	0.5166
Reduced model	-81.8319	24.3516	3	<.0001

AIC: 143.591

This indicates model fit is not statistically significant

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	49	0
9.6700	0.0671	3.290	6	49	1.547
29.0000	0.1881	9.030	8	48	-0.3804
58.0000	0.3409	16.361	15	48	-0.4145

Chi-square = 2.71 DF = 3 P-value = 0.4384

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 14.6608 **Estimated LED_{10}**

BMDL = 10.9472

Attachment E
BMD Analysis, Quantal Quadratic, Full Data Set

```
 ================================================================
        Quantal Quadratic Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
        Input Data File: D:\MYSAS\CALEPA\NAPHTHALENE.(d)
        Gnuplot Plotting File:  D:\MYSAS\CALEPA\NAPHTHALENE.plt
                                          Sat Feb 28 09:42:05 2004
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]


   Dependent variable = COLUMN2
   Independent variable = COLUMN1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =         0.01
                          Slope =  0.000110055
                          Power =            2   Specified


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Power
                  have been estimated at a boundary point, or have been specified by
the user,
                  and do not appear in the correlation matrix )

              Background        Slope

Background            1        -0.49

     Slope        -0.49            1
```

Attachment E (cont)
BMD Analysis, Quantal Quadratic, Full Data Set

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.056394	0.0258121
Slope	0.000105952	3.24488e-005

All model terms fitted

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-69.6561			
Fitted model	-73.7905	8.26872	2	0.01601
Reduced model	-81.8319	24.3516	3	<.0001

AIC: 151.581

This indicates model fit is statistically significant

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0564	2.763	0	49	-1.711
9.6700	0.0657	3.219	6	49	1.603
29.0000	0.1368	6.568	8	48	0.6013
58.0000	0.3393	16.287	15	48	-0.3923

Chi-square = 6.02 DF = 2 P-value = 0.0494

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 31.5343

BMDL = 25.2981 **Estimated LED_{10}**

Attachment F
MSTAGE Output, Full Data Set